

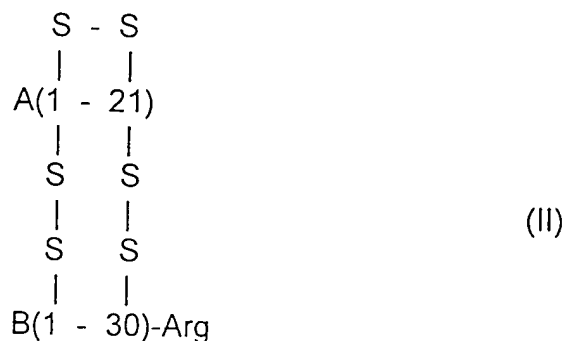
APPENDIX B
PENDING CLAIMS

U.S. Patent Application No. 08/402,394

Filed: March 10, 1995

Inventors: Michael DORSCHUG et al.

21. (Thrice Amended) A method for the preparation of a mono-Arg-insulin compound of formula II



in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which comprises:

(a) expressing in a bacterium a DNA molecule encoding a fusion protein which comprises a mini-proinsulin compound of the formula:



- (b) liberating said mini-proinsulin compound from said fusion protein;
- (c) folding and forming disulfide bridges in said mini-proinsulin compound;
- (d) incubating said mini-proinsulin compound with trypsin; and
- (e) precipitating the mono-Arg-insulin.

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22. (Twice Amended) A method for the preparation of insulin which comprises:

(a) expressing in a bacterium a DNA molecule encoding a fusion protein

which comprises a mini-proinsulin compound of the formula:



in which B(1-30) and A(1-21) denote the B and A chains of insulin;

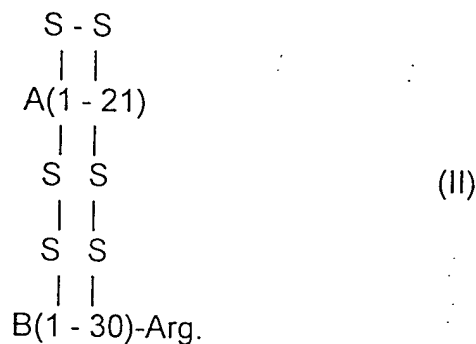
(b) liberating said mini-proinsulin compound from said fusion protein;

(c) folding and forming disulfide bridges in said mini-proinsulin compound;

(d) simultaneously incubating said mini-proinsulin compound with trypsin and carboxypeptidase B; and

(e) precipitating the insulin.

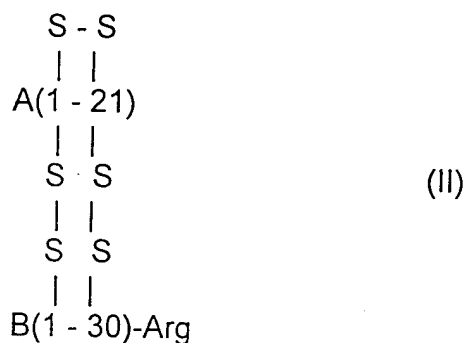
23. A method as claimed in claim 22, wherein step (d) is carried out in one vessel without having to isolate as an intermediate mono-Arg-insulin of formula II



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25. (Amended) A method for the preparation of a mono-Arg-insulin compound of formula II



in which A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, which comprises:

(a) expressing in a bacterium a DNA molecule encoding a fusion protein which comprises



bonded via a bridging member,



to a peptide which stabilizes the fusion protein;

(b) liberating a mini-proinsulin compound from said fusion protein by cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide;

(c) folding and forming disulfide bridges in said mini-proinsulin compound;

(d) incubating said mini-proinsulin compound with trypsin; and

(e) precipitating the mono-Arg-insulin.

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26. (Amended) A method for the preparation of insulin which comprises:

(a) expressing in a bacterium a DNA molecule encoding a fusion protein which comprises

B(1-30)-Arg-A(1-21)

bonded via a bridging member,

- Met - Ile - Glu - Gly -Arg -,

to a peptide which stabilizes the fusion protein;

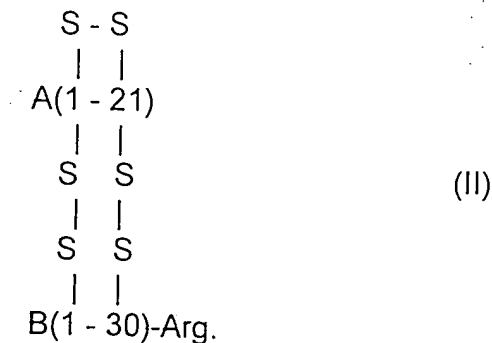
(b) liberating a mini-proinsulin compound from said fusion protein by cleaving the expressed fusion protein resulting from step (a) with cyanogen bromide;

(c) folding and forming disulfide bridges in said mini-proinsulin compound;

(d) simultaneously incubating said mini-proinsulin compound with trypsin and carboxypeptidase B; and

(e) precipitating the insulin.

27. A method as claimed in claim 26, wherein step (d) is carried out in one vessel without having to isolate as an intermediate mono-Arg-insulin of the formula II



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31. (Amended) A method for the preparation of insulin, without formation of substantial amounts of insulin Des-B30, comprising:

(a) expressing in a bacterium a DNA molecule encoding a fusion protein which comprises

B(1-30) - Arg - A(1-21)

bonded via a bridging member,

-Met-Ile-Glu-Gly-Arg-,

to a peptide which stabilizes the fusion protein;

(b) liberating a mini-proinsulin compound from said fusion protein by cleaving the expressed fusion resulting from step (a) with cyanogen bromide to produce mini-proinsulin;

(c) incubating the product formed in step (b) with sodium tetrathionate to form hexa-5-sulfonate;

(d) simultaneously incubating the S-sulfonate mini-proinsulin formed in step (c) with trypsin and carboxypeptidase; and

(e) precipitating the insulin.

33. A compound of the formula I

B(1-30)-Arg-A(1-21) (I)

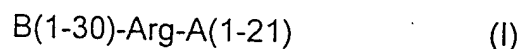
wherein A(1-21) and B(1-30) denote the A and B chains of human insulin.

34. A nucleic acid sequence encoding the compound of formula I as claimed in claim 33.

35. A vector comprising the nucleic acid sequence of claim 34.

36. A host cell containing the nucleic acid sequence of claim 34.

37. A fusion protein comprising a compound of the formula I



wherein A(1-21) and B(1-30) denote the A and B chains of human insulin, and wherein the compound is bonded via a bridging member



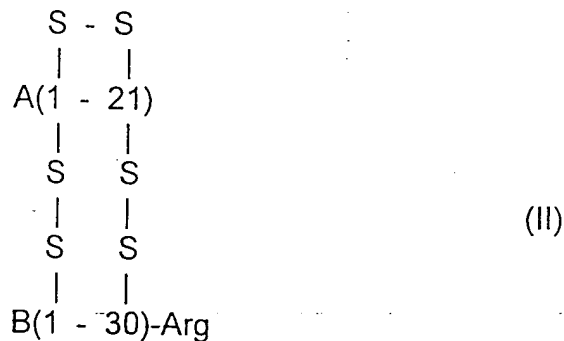
to a peptide which stabilizes the fusion protein.

38. A process for the preparation a compound as claimed in claim 33, which comprises:

a) expressing a DNA sequence encoding the compound of the formula I in a bacterium; and

b) when the DNA sequence encodes a fusion protein, liberating the compound of formula I from the fusion protein.

39. A method for the preparation of a compound of the formula II

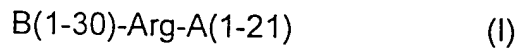


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wherein A(1-21) and B(1-30) denote the A and B chains of human insulin and the -S-S- bridges are positioned as in insulin, comprising:

- a) expressing a DNA sequence encoding the compound of formula I

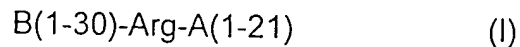


in a bacterium; and

- b) cleaving the expressed compound of step (a) with trypsin.

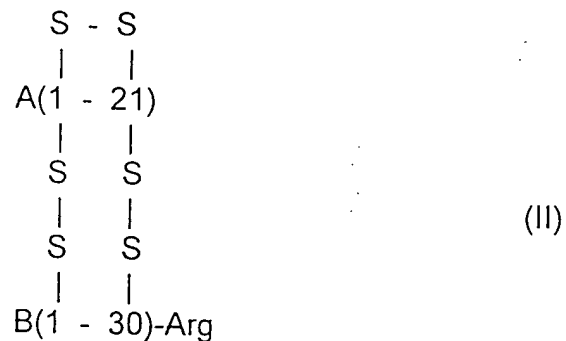
40. (As Amended on October 19, 1999) A method for the preparation of insulin comprising:

- a) expressing a DNA sequence encoding the compound of formula I



in a bacterium;

- b) cleaving the expressed compound of step (a) with trypsin resulting in the compound of the formula II



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